PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference LPB/P103622WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2005/000015	International filing date *(day/month/year)* 06.01.2005	Priority date *(day/month/year)* 06.01.2004

International Patent Classification (IPC) or national classification and IPC
INV. G01N33/96 G01N33/68

Applicant
BADRILLA LIMITED et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.11.2005	27.04.2006

Name and mailing address of the international preliminary examining authority:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized officer

Routledge, B

Telephone No. +31 70 340-4272

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/000015

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-40 as originally filed

   Claims, Numbers

   1-56 received on 22.03.2006 with letter of 21.03.2006

   Drawings, Sheets

   1/12-12/12 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/000015

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-56
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-56
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-56
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in electronic form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: BROCKWELL ET AL: "The effect of core destabilisation on the mechanical resistance of I27" BIOPHYSICAL JOURNAL, vol. 83, July 2002 (2002-07), pages 458-472,

1. The filed amendments meet the requirements of Article 34(b) PCT.

2. The present claims relate to a presentation system comprising an extremely large number of possible target moieties and scaffolds and combinations thereof. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the target moiety/scaffold combinations. The only subject matter meeting the conditions of Articles 5 and 6 PCT would appear to be the use of A1, PS38 (SERCA2a) or PT17 as target moiety and scaffold proteins comprising the I27 or I39 domain(s)

3. Notwithstanding the above lack of support and disclosure, the subject matter of claims **1-56** meets the requirements of Article 33(2) and (3) PCT as the use of said presentation system and methods for quantifying the amount of target moiety in a sample using the presentation system as claimed are not disclosed or suggested in the cited prior art which either teach the use of said systems for a different unrelated purpose (D1) or do not clearly establish the controllable nature of the system

3.1. The closest prior art is **D1** (page **459**) which discloses a target moiety (fluorophore) attached to a domain on a scaffold (the I27 concatamer via a central C63S domain). As is clearly demonstrated by **D1**, the number of I27 scaffold domains may be "controlled" by the simple choice of the number of cassettes ligated together. However this feature is merely descriptive of the scaffold material and does not

represent a distinguishing technical feature which in the present application lies in the use to which the system is put. **D1** is concerned with the investigation of the dynamics of protein folding and unfolding and does not teach or suggest the use of the disclosed system for quantitative determination or calibration purposes.

4. All claims meet the criteria of Article 33(4) PCT with regard to industrial applicability.

CLAIMS

1. Use of a non-natural presentation system in quantifying an amount of a target moiety which is present in a sample, the presentation system comprising at least one copy of a target moiety or part thereof that is recognisable by a binding partner and at least one domain of a scaffold material covalently linked to said target moiety wherein the scaffold material has a controllable property, and wherein the at least one domain of the scaffold is non-reactive to a binding partner specific to said target moiety or part thereof.

2. Use according to claim 1 wherein the copy of the target moiety or part thereof is selected from the group comprising sequences of DNA or RNA, a peptide, an antigenic structure or a chemical entity or moiety.

3. Use according to either preceding claim wherein the target moiety further includes saccharides, metabolite cofactors, haptens or a modification by a phosphate, nitrosylated group, sulphated group or glycosylphosphatidyl inositol (GPI) group.

4. Use according to any preceding claim wherein the controllable property is relative molecular mass (Mr) or weight (Mwt) or isoelectric point (pI).

5. Use according to any preceding claim wherein the scaffold material is a protein.

6. Use according to any preceding claim wherein the scaffold material comprises at least one natural or unnatural amino acid with at least one or more chemically reactive groups within a side chain of a residue.

7. Use according to claim 6 wherein the chemically reactive acidic group is selected from the group comprising a carbonyl on glutamic acid, aspartic acid, a hydroxyl on tyrosine, a thiol on cysteine and a primary amine on lysine.

8. Use according to any preceding claim wherein the scaffold comprises one or more chemically reactive cysteine and/or lysine amino acid residues.

9. Use according to claim 8 wherein the number of reactive cysteine and/or lysine residues is controlled by either selecting the scaffold protein from a natural source which contains a desired number of reactive cysteine and/or lysine groups or by selectively

mutating in or out a reactive reside from a protein sequence or rendering ineffective any one or more of the reactive cysteine and/or lysine residues of a selected scaffold protein.

10. Use according to any preceding claim wherein the scaffold is selected from the group comprising: I27 domain from titin; I39 domain which is a subunit (subunit 5) of splicing factor 3b; organ of Corti protein (Mus musculus); heat shock protein, mitochondrial (Mus musculus); splicing factor 3B subunit 5 (Mus musculus); ubiquinol-cytochrome C reductase complex ubiquinone-binding protein; E1B protein (Human adenovirus type 11); chaperonin (Arabidopsis thaliana); photosystem II reaction center H protein (Arabidopsis thaliana); a NADH-ubiquinone oxidoreductase subunit, mitochondrial [Precursor] (Homo sapiens); signal recognition particle protein (Mus musculus); and DNA polymerase delta subunit 4(Mus musculus).

11. Use according to claim 10 comprising a combination of any one or more of the scaffolds of claim 10.

12. Use according to claim 10 comprising a plurality of identical scaffolds.

13. Use according to claim 10 claim comprising a plurality of identical or non-identical domains as a mixture or blend thereof.

14. Use according to claim 10 comprising a plurality of scaffold I27 domains from titin and wherein where at least one domain or unit is engineered to possess a single cysteine residue for peptide attachment while all other or a selected number of I27 domain or unit lacks cysteine residue(s) or other reactive residues selected from the group comprising lysine, glutamate and aspartate.

15. Use according to claim 10 comprising at least one or more scaffold I39 domain(s) which is/are a subunit (subunit 5) of splicing factor 3b.

16. Use according to any preceding claim wherein the scaffold domains are about 10kDa.

17. Use according to any preceding claim wherein the scaffold linear units or domains are linked in tandem.

18. Use according to any preceding claim comprising at least one biological or non-biological polymer.

19. Use according to claim 18 wherein the non-biological polymer is PEG (polyethylene glycol).

20. Use according to any preceding claim wherein the copy of the target moiety is incorporated into the presentation system using either covalent attachment through a thiol group on a cysteine residue or in the instance of the target moiety being a protein or peptide and the presentation system is also a protein or peptide, a DNA segment encoding the target moiety is incorporated into the DNA encoding the presentation system, and subsequently expressed continuous with the presentation system.

21. Use according to any preceding claim wherein the domains of the presentation system, apart from the copy of the target moiety or part thereof, are substantially inert or are non-reactive to the specific binding partner of the sample target moiety or part thereof.

22. Use according to any preceding claim wherein the copy of the target moiety or part thereof is not identical to the target moiety present in the sample.

23. Use according to any preceding claim comprising a plurality of copies of the target moieties.

24. Use according to any preceding claim wherein the copy of the target moiety or part thereof is linear or branched within the presentation system.

25. Use according to claim 24 wherein the copy of the target moiety is branched so that the covalent attachment is via a side chain of the scaffold material.

26. Use according to any preceding claim wherein the binding partner is selected from the group comprising monoclonal antibodies, polyclonal antibodies, RNA or DNA or peptide aptamers or other antibody equivalents, dyes, drugs and metal chelates.

27. Use according to any preceding claim comprising at least one I27 domain and/or at least one I39 domain and a target moiety selected from the group comprising A1, PS-38 or PT17 peptide.

28. Use of a product in quantifying the amount of a target moiety which may be present in a sample, the product comprising a plurality of presentation systems, each presentation system comprising at least one copy of a target moiety or part thereof and at least one domain covalently linked to said copy of the target moiety, wherein the domain(s) is/ are non-reactive to a binding partner specific to said copy of the target moiety or part thereof, further wherein each presentation system has a different molecular weight from other presentation systems in the product.

29. Use according to any preceding claim of the presentation system as a positive control or internal standard or in generating a calibration curve.

30. A kit for quantifying the amount of a target moiety in a sample, the kit comprising a presentation system as defined in any of claims 1 to 27.

31. A method of quantifying the amount of target moiety in a sample which may contain the target moiety, the method comprising:

a) providing a presentation system which comprises at least one copy of the target moiety or part thereof that is recognisable by a binding partner and at least one domain which is non-reactive to said binding partner, said at least one copy of the target moiety being covalently bonded to the at least one domain of a scaffold material that has a controllable property;

b) carrying out a separation detection technique on said presentation system, wherein said presentation system is present in a specific amount;

c) generating at least one comparison point comprising intensity of a signal produced by the presentation system versus the amount of the presentation system.

32. A method according to claim 31 wherein the presentation system is present in a single specific amount.

33. A method according to claim 31 wherein the presentation system is present in a series of varying amounts.

34. A method according to claim 33 wherein the varying amounts are in the same or different lanes or channels of a blot.

35. A method according to either of claims 33 or 34, wherein the comparison point is a plurality of comparison points which together provide a calibration curve.

36. A method according to any of claims 33 to 35 claim further comprising comparing the comparison point or comparison points with the sample to quantify the amount of target moiety present in the sample.

37. A method according to any of claims 31 to 36, wherein said presentation system is of a known molecular weight or pI.

38. A method according to any of claims 33 to 39, wherein the presentation system comprises a non-biological polymer, a nucleic acid molecule, a peptide, protein or combinations thereof.

39. A method according to any of claims 31 to 38, wherein the presentation system comprises a plurality of domains linked in tandem.

40. A method according to any of claims 31 to 39, wherein the presentation system comprises identical units or domains or non-identical or different units or domains.

41. A method according to any of claims 31 to 40 wherein the unit(s) of the presentation system is/are non-reactive to the binding partner specific to the target moiety of part thereof.

42. A method according to any of claims 31 to 41 wherein the copy of the target moiety or part thereof comprises sequences of DNA, RNA, protein or peptide, saccharides, haptens, phosphate, nitrosylated groups, sulphated groups, GPI groups, an epitope, an antigenic structure or a chemical entity.

43. A method according to claim 42 wherein the copy of the target moiety comprises SERCA2a or SERCA2a phosphorylated on serine-38.

44. A method according to any of claims 31 to 43 wherein the presentation system comprises differing target moieties or parts thereof.

45. A method according to any of claims 31 to 44, wherein the copy of the target moiety or part thereof is linear or branched within the presentation system.

46. A method according to any of claims 31 to 45, wherein the specific binding partner comprises a molecule which has a specific binding affinity for the target moiety and is capable of binding thereto.

47. A method according to claim 46 wherein the binding partner comprises an antibody, DNA sequence, RNA sequence, a polypeptide, a dye, a metal chelate or a drug molecule.

48. A method according to any of claims 31 to 47, wherein the separation based detection technique comprises a dot blot, Western blot, RIA, fluorescence polarisation, ELISA, Northern blotting, Southern blotting, PCR, High Performance Liquid Chromatography (HPLC), capilliary electrophoresis, 1D electrophoresis, isoelectric focusing, mass spectrometry or combinations of the above.

49. A method according to any of claims 31 to 48 wherein the presentation system acts as a positive control for detecting the presence or absence of a target moiety in a sample.

50. A method according to any of claims 31 to 48 wherein the presentation system acts as an internal standard by providing a one point calibration.

51. A method according to any of claims 31 to 48 wherein the presentation system is used to generate multiple comparison points so as to provide a calibration curve.

52. A method according to any of claims 31 to 48 wherein the presentation system is used to monitor efficiency of immunoprecipitation and/or stages of an immunoprecipitaion process.

53. A method according to any of claims 31 to 52 further including any one or more of the features of claims 2 to 27.

54. A method for quantifying an amount of a protein epitope in a sample, said method comprising:

(a) providing a protein presentation system comprising at least one copy of the protein epitope and at least one further protein domain, wherein said presentation system is of known molecular weight;

b) carrying out a Western blot experiment on said presentation system, wherein said presentation system is in a specific concentration; wherein said Western blot experiment utilises a binding partner specific to the target moiety; and further wherein said protein domain of the presentation system is non-reactive to the binding partner; and

c) generating a comparison point comprising intensity of a signal produced by the presentation system in said technique versus the concentration of the presentation system.

55. A method according to any of claim 54 further including any one or more of the features of claims 2 to 27.

56. A product comprising at least one copy of a target moiety or part thereof that is recognisable by a binding partner and at least one domain of a scaffold material covalently linked to said target moiety wherein the scaffold material has a controllable property, and wherein the at least one domain of the scaffold is non-reactive to a binding partner specific to said target moiety or part thereof, the target moiety being selected from the group comprising A1, PS-38 or PT17 peptides and the scaffold material being selected from the group comprising: an I27 domain from titin; I39 domain which is a subunit (subunit 5) of splicing factor 3b; organ of Corti protein (Mus musculus); heat shock protein, mitochondrial (Mus musculus); splicing factor 3B subunit 5 (Mus musculus); ubiquinol-cytochrome C reductase complex ubiquinone-binding protein; E1B protein (Human adenovirus type 11); chaperonin (Arabidopsis thaliana); photosystem II reaction center H protein (Arabidopsis thaliana); a NADH-ubiquinone oxidoreductase subunit, mitochondrial [Precursor] (Homo sapiens); signal recognition particle protein (Mus musculus); and DNA polymerase delta subunit 4(Mus musculus). .